ILLICIUM FLORIDANUM, ELLIS.

HISTOLOGICAL AND CHEMICAL EXAMINATION.

THESIS PRESENTED TO THE PHILADELPHIA COLLEGE OF PHARMACY, MARCH, 1885,

FOR THE DEGREE OF GRADUATE IN PHARMACY. (PH.G.)

BY

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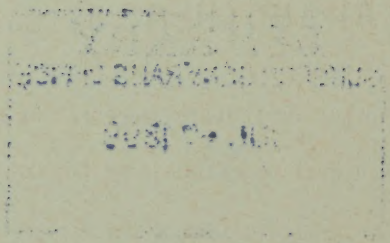
"MERRIHEW PRINT" (J. SPENCER SMITH), 501 CHESTNUT ST., PHILA.



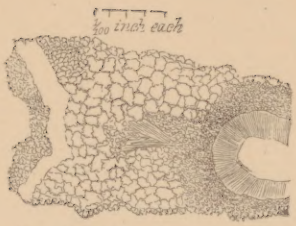


FIG. 14.—Capsule: transverse section through dorsal suture.

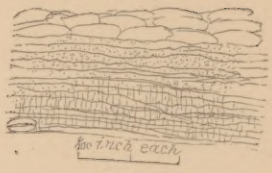


FIG. 15.—Capsule: longitudinal section.

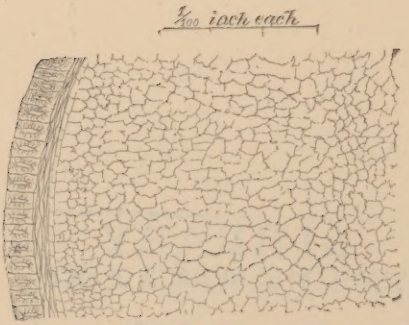


FIG. 17.—Seed: transverse section.

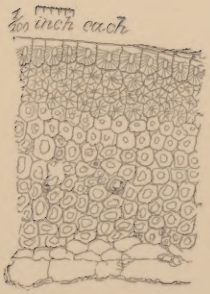


FIG. 16.—Capsule: transverse section near ventral suture.

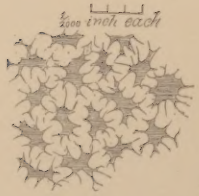


FIG. 18.
Testa: exterior view.

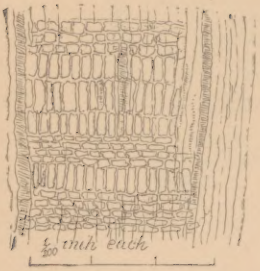


FIG. 7.—Stem: radial section.

FIG. 8.—Stem: tangential section

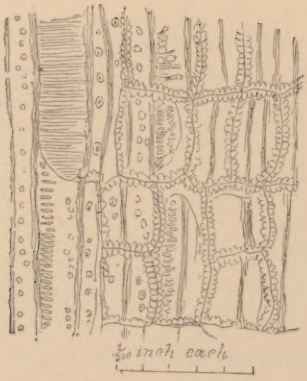


FIG. 9.—Stem: radial section.

FIG. 10.—Epidermis of leaf.

FIG. 11.—Stoma.

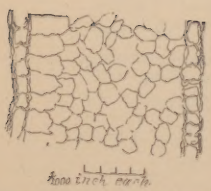


FIG. 12.—Leaf: transverse section, with stoma on lower surface.

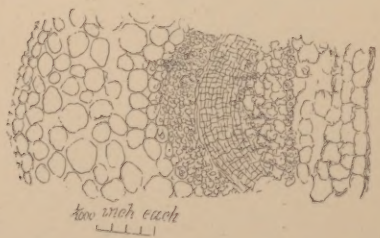


FIG. 13.—Leaf: transverse section, through midrib.

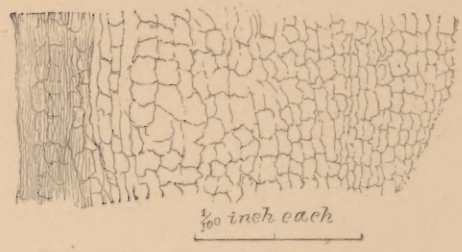


FIG. 4.—Stem-bark: longitudinal section.

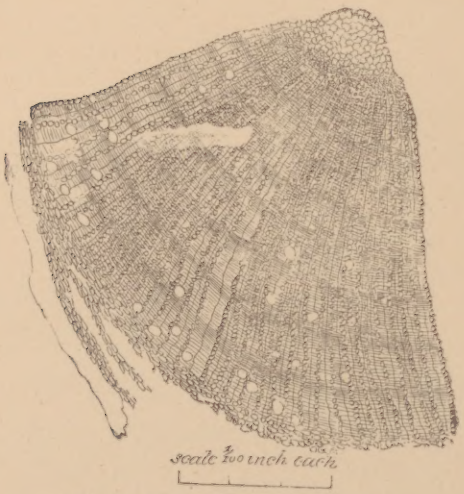


FIG. 5.—Stem: transverse section.

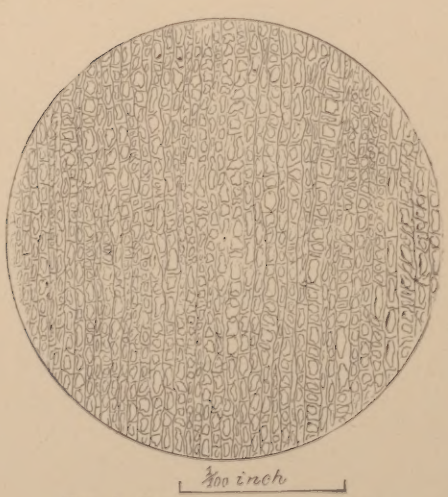


FIG. 6.—Stem: transverse section.

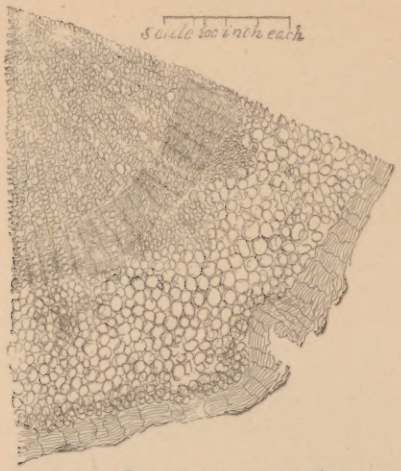


FIG. 1.—Root: transverse section.

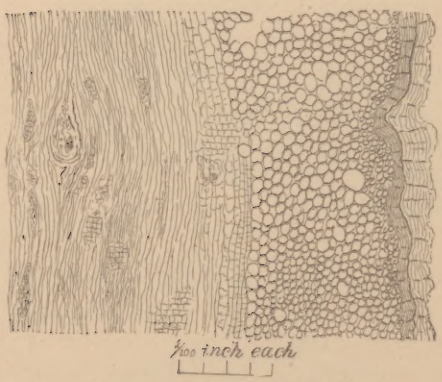


FIG. 2.—Root: longitudinal section.

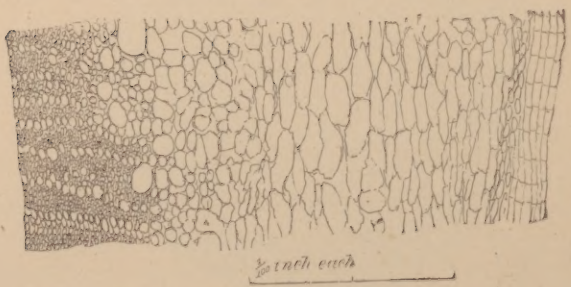


FIG. 3.—Stem-bark: transverse section.

[Reprinted from the American Journal of Pharmacy, May and June, 1885.]

ILLICIUM FLORIDANUM

(ELLIS).

Natural order, Magnoliaceæ Illicieæ.

BY HENRY C. C. MAISCH, PH.G.

This species is a shrub or small tree growing in swamps in Florida, Alabama and westward to Mississippi, and is popularly known as Southern star anise, Florida stink bush, or poison bay.

The leaves are about 4 inches long and 1½ inch wide, short petiolate, acuminate, alternate, oblong-lanceolate, entire, smooth, indistinctly pellucid punctate. The midrib is very prominent on the lower side. Flowers are about one inch in diameter; sepals six, green gradually changing to color of petals, deciduous; petals purplish crimson or dark purple, linear, obtuse, in three whorls, the outer having often the color of the sepals; stamens 30 or more, about ½ inch long and ⅛ as wide; the anthers consist of two cells adnate, introrse, half the length of the stamen, dehiscence longitudinal; ovaries 13, separate; styles short, erect. In the flowers the carpels are erect, but after fructification they spread horizontally, forming a right angle with the axis. The carpels are capsular, dry, not woody, dehiscent at the ventral suture, and contain each one smooth shining seed about the size of an apple seed.

Griffith (*Med. Botany*, p. 101) said that the bark may be used as a substitute for cascarilla. The leaves are poisonous. The material for this investigation was kindly furnished by Hiland Flowers, Ph.G., of Amite City, La. (now of New Orleans).

HISTOLOGICAL INVESTIGATION.

Root.—The root, on cross-section, shows the bark and the wood to be of about the same thickness and also the entire absence of pith. The cork cells (Figs. 1 and 2) are flattened and tangentially elongated.

The bark is composed of large and small thin-walled parenchymatous cells, the small ones being near the cambium and constituting the inner bark, through which the medullary rays pass. The cells of the rays are larger than the surrounding bast parenchyma, and in the outer liber layer gradually increase in size to that of the cells in the primary or outer bark. The secondary bark contains no bast fibres and is made up of about 12 layers of cells. The primary bark is composed of about 24 layers, the cells increasing in size as they approach the middle. The cambium layer consists of about three rows of minute cells. The wood contains numerous medullary rays, one or two cells wide and from five to twenty cells high in an axial direction. It furthermore contains wood parenchyma, ducts and prosenchyma. The cells of the first are somewhat thickened, are axially elongated and found between the groups of medullary rays. The ducts are thinner-walled and larger than the prosenchyma, and have spiral and scalariform markings, while the prosenchyma cells are quadrangular and are marked by dots and disks.

Stem.—The bark is made up of two distinct layers (Figs. 3 and 4) besides the cork. The cork cells are flattened, closely packed, and elongated in a tangential direction. The primary bark is composed of large, thin-walled cells, which on cross-section are hexagonal and tangentially elongated. The inner layer of the primary bark has two or three interrupted circles of oil cells, the rest being smaller and hexagonal. The outer layer of the primary bark is composed of hexagonal, tangentially elongated and large cells. The secondary bark is composed of a layer of about twenty bast parenchyma cells and twelve cells of the medullary rays, these latter being larger and the secondary deposit relatively thinner than in the former cells, these being axially elongated. The cambium layer is about three cells in thickness. (Figs. 5 and 6.) The wood consists of medullary rays, wood parenchyma, ducts and prosenchyma. The medullary rays, on a cross-section, are seen to be made up of small radially elongated cells, the walls of which are materially thickened by secondary deposit, and which are placed in one or two radial rows, each row containing, in an axial direction, from three to seventeen cells. The wood parenchyma is only found between the perpendicular ends of the medullary rays, consequently in axial lines. (Figs. 7 and 8.) The cells are axially elongated, the cell walls are considerably thickened. The ducts have netted, spiral and scalariform markings (Fig. 9), while the lateral sides of the prosenchyma cells are

marked with dots and disks. The pith is composed of hexagonal cells on a cross-section. The cells are smallest near the wood, and gradually enlarge toward the centre.

Leaf.—The epidermis is composed of a single layer of flattened, thick-walled empty cells. The stomata are very numerous on the lower surface (Fig. 10), while only one was found in a space $\frac{3}{8}$ inch square of the upper surface. They are made up of four cells, two inner and two outer guard cells. (Fig. 11.) On a cross-section, the inner guard cells are seen to bulge into the opening at about half the height of the cells. The outer guard cells are slightly larger and overlap the inner cells at the edges. The rest of the epidermal cells have wavy cell walls. The portion of the leaf between the epidermal layers is made up of loose parenchyma with large air passages and a single palisade layer occupying (Fig. 12) about one-fourth the thickness of the leaf. The midrib is composed of wood fibres, which, on cross-section, appear quadrangular with their cell walls slightly thickened (Fig. 13). This prosenchymatous tissue is arranged in radiating lines and terminates above and below by curves. The concavity, which is toward the upper side of the leaf, is filled by the large-celled spiral ducts, and above this is a single row of bast fibres. On the lower side, the wood is surrounded by a cambium layer and outside of this there are two or three layers of bast fibres. Surrounding this bundle is the loose parenchyma tissue of the leaf, there being three layers of small cells above, and about six to eight layers below, the cells as they approach the epidermis becoming thick-walled. The upper and lower surface of the leaf at this point is covered by a rather thick cuticle.

Capsule.—The capsules are composed mainly of parenchymatous tissue, there being only one unbranched fibrovascular bundle in each carpel running through the fleshy portion at the dorsal suture (Figs. 14 and 15). The receptacle for the seed is lined with closely packed elongated thick-walled cells having one end directed toward the seed. The cells near the ventral suture are in three layers (Fig. 16), the outer layer being composed of large parenchyma cells which are elongated in the direction of the style. The other two layers contain axially elongated cells with thickened cell walls, those of the middle layer having the smaller deposit. The outer layer is made up of about four rows of cells and the other two consist of about ten rows together.

Seed.—The seeds are albuminous. The albumen consists of hex-

agonal cells, seen in the section (Fig. 17), and contain oil. The endopleura consists of one layer of cells, the walls of which are somewhat thickened. The testa consists of two layers, the inner is composed of about three rows of cells with thickened walls (Figs. 17 and 18), and the outer layer is made up of a single row of cells. The cell walls of the outer layer of the testa are very much thickened and extend in a radial direction. The exterior view of the testa shows the cell walls as wavy lines. The external layer of the testa is yellowish, while the interior one is brown.

CHEMICAL INVESTIGATION.

The analytical researches were made in the chemical laboratory of the Philadelphia College of Pharmacy. The parts of the plant were taken in No. 80 powder, and the estimations were made on the plant as obtained and not previously dried by artificial means.

Leaves.—The moisture of the leaves was determined to be 13·75 per cent. The same portion was used for estimating the ash which amounted to 5·033 per cent. The solubilities of the ash were as follows:

Soluble in water	1·600
Soluble in hydrochloric acid	2·500
Insoluble in either (silica)	0·933
	5·033 per cent.

The qualitative analysis resulted as follows: Acids, carbonic and phosphoric; Bases, potassium, sodium, aluminium, iron and calcium.

1. Petroleum extraction.—A portion of the powder was macerated with petroleum spirit (boiling point below 45°C.), the quantity being 1 gm. to 10 cc., which proportion was retained throughout the whole analysis. The percentage of residue remaining on spontaneous evaporation of a portion of the liquid was found to be 2·60, of which, on being heated, 0·23 was lost. This loss was estimated as volatile oil, because, on heating, the extract became entirely odorless. The reactions of this volatile oil are given under "Capsules," as the yield there was greater. The extract was not saponified by either aqueous or alcoholic solution of potassium hydrate. The latter solution was precipitated by the subsequent addition of water. The odorless residue amounted to 2·37 per cent.

2. Ether extraction.—The powder was next treated with the requisite quantity of ether. A part of the resulting liquid was evaporated and

the residue found to amount to 1·46 per cent., which lost nothing on heating to 110°C. The residue from another portion was treated with water, which acquired a bitter taste; the dissolved principle will be treated under the alcoholic maceration as this dissolved a larger quantity. The residue from the ethereal solution was completely soluble in strong alcohol, chloroform, benzol, aqueous and alcoholic potassium hydrate solution. The alcoholic solution was precipitated by distilled water and also by acidulated water. The extract was principally resin.

3. Absolute Alcohol maceration.—The residual powder from the above maceration was treated with the necessary quantity of absolute alcohol. The dried extract was equal to 5 per cent., which lost nothing on heating to 110°C. The air-dried residue was treated with water heated slightly and allowed to macerate for 24 hours. The water had assumed an acid reaction and a yellow color which was turned to a yellowish brown by ammonia water. The portion soluble in water amounted to 4·29 per cent. The aqueous solution was acidified and shaken with petroleum, benzol and chloroform. The liquid was afterward made alkaline and shaken with the same liquids, ether being used in addition. Most of the residues were crystalline.

In order to obtain a larger quantity of the crystals 75 grams of the drug were treated with 95 per cent. alcohol. This tincture was evaporated to a small bulk and precipitated with water. The filtrate obtained was shaken with petroleum, benzol and chloroform. The residue from the petroleum was entirely soluble in aqueous sodium hydrate solution, and after neutralization with hydrochloric acid a precipitate of a brownish color formed. The aqueous solution was then acidified and shaken with the liquids mentioned above. The residues from benzol and chloroform had a bitter taste, a neutral reaction and a crystalline structure. The same crystals were also found after the acidified aqueous liquid had been made alkaline and at that place the reactions are also given.

The precipitate obtained from the alcohol was boiled with dilute acid and this liquid shaken as above, and also after it was made alkaline. The residues consisted of a yellow resin neutral to test paper.

The undissolved precipitate from above was boiled with potassium hydrate solution and this shaken with the same liquids noticed above. The residue from the petroleum shaking consisted of a soft resinous mass and of colorless crystalline plates, the whole having the odor of orris root. The crystals were freed from adhering resin by a few

drops of alcohol. This liquid, like the dry resin, gave a green color with sulphuric, hydrochloric and nitric acids, the degree of intensity being in the order as enumerated, sulphuric acid giving the stronger color. Sodium hydrate dissolves but does not saponify the resin.

The crystals gave no precipitates with alkaloidal reagents, and did not produce any ammonia on heating with potassium hydrate. After boiling with dilute acid, they reduced alkaline copper solutions. The following are the reactions obtained for this principle: Sulphuric acid dissolved it in the cold without color, but on warming, the color became carmine-red. It gave no reaction with hydrochloric acid or nitric acid. Sulphuric acid and bichromate of potassium (Otto's test) gave in the cold a brownish yellow color, which on heating was changed to green. Sugar and sulphuric acid (Schneider's test) gave no color in the cold, but on warming the color is first light brown and rspidly darkens by the caramelizing of the sugar, while all through the reaction red spots are visible. Frochde's test (molybdate of ammonium and sulphuric acid) gave first a green color, and on heating slightly the color was rapidly changed to a dark blue. These reactions show the principle to be different from Eykman's sikimin, which does not reduce Fehling's test, even after boiling with dilute acid. From the reactions noted above, the crystals were regarded as those of a glucoside to which the bitter taste of the leaves is due. The crystals amounted to about 0·75 per cent.

The residues from benzol and chloroform shakings were of a dark green color and soluble in alcohol. The alcoholic solution was clouded by the addition of water, and further by a few drops of hydrochloric acid. The same solution gave with sulphuric acid a green liquid and a brown precipitate. The same reaction was obtained with hydrochloric and nitric acids; but, if heat be applied to the test with sulphuric acid, it becomes of a purplish color and ultimately chars; if the liquid with nitric acid is treated in the same manner, the precipitate becomes yellow and the green color is discharged; if to the hydrochloric acid test a few drops of nitric acid be added, the color is changed to red and ultimately to red-brown. If a quantity of the dry extract (resin) be heated in a glass tube open at both ends, it gives a reddish brown sublimate, inflammable vapors and a residue of charcoal. The shakings of the acidified alkaline solution gave nothing but resin.

4. Aqueous Extraction.—The powder from the above maceration

was treated with the required quantity of water which formed a thick mucilage; it was therefore diluted to allow of straining, and by evaporation brought to the first quantity when the liquid was thinner and permitted filtration. There was about 6·335 per cent. of precipitate present, which was regarded as albuminous matter. The liquid was then treated with twice its quantity of 95 per cent alcohol, which gave a precipitate amounting to 4·75 per cent. The precipitate from the filtrate by lead acetate was yellowish brown in color. This was separated, suspended in water and decomposed by hydrogen sulphide. The filtrate from this precipitation was evaporated to drive off hydrogen sulphide, this liquid then gave precipitates with barium and calcium hydrates, and a green color with ferric chloride; it was also precipitated by gelatin solution, by which means it was estimated. The tannin present amounted to 3·96 per cent.

5. Alkaline Extraction.—The powder was next treated with a 0·2 per cent. solution of sodium hydrate. The dissolved portion amounted to 9·9 per cent.

6. Acid Maceration.—The insoluble portion of the leaves was then treated with diluted 2 per cent. hydrochloric acid. The extract amounted to about 6 per cent.

The remaining, undissolved portion was bleached and weighed. It amounted to 42·6 per cent. This was regarded as lignin.

Stem.—The moisture and ash of the stem, as in the case of the leaves, were estimated from the same portion of the powder. The moisture amounted to 10·16 per cent. and the ash to 1·333 per cent., the small amount of ash being probably due to the large amount of sclerenchyma.

The solubilities of the ash are as follows:

Soluble in water.	·033
Soluble in hydrochloric acid.....	1·067
Insoluble in either.....	·233
	1·333 per cent.

The qualitative analysis gave the following result: Acids, sulphuric and hydrochloric; Bases, magnesium, potassium, sodium, iron.

1. Petroleum maceration.—The powder was macerted with the necessary quantity of petroleum, the portion soluble herein amounted to 0·19 per cent. which lost nothing on heating to 110°C. The residue was soluble in ether, tasteless, unsaponifiable by either alcoholic or aqueous solution of potassium hydrate.

2. Ether extraction.—The powder was next treated with ether. The portion soluble in ether amounted to 0·23 per cent. The extract was soluble in alcohol, and in aqueous and alcoholic solution of sodium hydrate. Its reactions show it to be an acid resin.

3. Absolute alcohol maceration.—Absolute alcohol was the next solvent used for maceration. The soluble portion amounted to 1·9 per cent., of which 1·687 was soluble in water, ·211 soluble in dilute ammonia, leaving ·002 as insoluble in either. The extract was astringent but not bitter. Tannic acid was present to the amount of 0·54 per cent.

4. Aqueous extraction.—The powder remaining from the foregoing macerations was treated with the necessary quantity of water. The precipitate obtained by the addition of alcohol amounted to 1·8 per cent. There was an acid present, but on account of decomposition of the liquid it could not be estimated, although the reactions show it to be tannic acid.

5. Alkaline extraction.—The powder was next treated with a 0·2 per cent. solution of sodium hydrate. A part of this solution was neutralized with acetic acid and subsequently alcohol added; the resulting precipitate amounted to 0·9 per cent; the total extract being equal to 5 per cent.

6. Acid maceration.—The powder insoluble in the above extractions was next treated with diluted hydrochloric acid of about 2 per cent. strength. A portion of this liquid was neutralized with ammonia. The resulting precipitate amounted to 0·24 per cent., the total extract amounting to 1·88 per cent. and containing some iron. The remaining insoluble powder after bleaching weighed 64·005 per cent.

Root bark.—The moisture of the bark amounted to 13·865 per cent. and the ash to 5·7 per cent. The solubilities of the ash were as follows:

Soluble in water	·334
Soluble in hydrochloric acid	2·833
Insoluble in either (silica)	2·533
	5·700 per cent.

The qualitative analysis resulted as follows: Acids: sulphuric and phosphoric; Bases: aluminium, calcium, magnesium, potassium, sodium.

1. Petroleum extraction.—The powder was extracted with petroleum as in the previous analyses. The soluble portion amounted to 2·60 per cent., of which 0·11 per cent. was volatile oil and the remainder 2·49 per cent. fat and a crystalline principle. The latter melts at 110°

C.; the same was also found in the capsules. The crystals were insoluble in alcohol and ether, but soluble in chloroform, and were neutral to test paper.

2. Ether maceration.—The powder was next treated with the necessary amount of ether. The extract amounted to 0·66 per cent., and consisted of resin.

3. Absolute alcohol treatment.—The powder was next treated with the requisite quantity of absolute alcohol. The soluble portion amounted to 12·2 per cent. The dry extract was soluble, as follows: 7·625 per cent. in water, 4·275 per cent. in dilute ammonia, and 0·3 per cent. insoluble in either. The aqueous solution contained tannin amounting to 5·4 per cent. The dilute ammonia dissolved the resin, which was of a ruby-red color and precipitable by neutralization with acetic acid.

4. Aqueous extraction.—The powder insoluble in the foregoing was treated with the necessary quantity of water. This mixture was diluted on account of the mucilage present and filtered. Alcohol gave a precipitate which amounted to 4 per cent., and on condensation alcohol precipitated 3 per cent. more. The total extract amounted to 8·96 per cent.

5. Maceration with diluted alkali.—The powder was next macerated with a 0·2 per cent. solution of sodium hydrate. The total extract amounted to 10·510 per cent., of which 9·605 per cent. was precipitated by alcohol and acetic acid to neutralization.

6. Treatment with diluted acid.—The powder insoluble in the foregoing extractions was treated with diluted 2 per cent. hydrochloric acid. The soluble portion amounted to 8 per cent. The remaining insoluble powder amounted to 42·625 per cent.

Capsules.—The moisture in the capsules amounted to 10·833 per cent. and the ash to 3·5 per cent. The solubilities of the ash were as follows:

Soluble in water	2·333
Soluble in hydrochloric acid	·467
Insoluble in either	.700
	3·500 per cent.

The qualitative analysis resulted as follows: Acids: sulphuric and phosphoric; Bases: copper, magnesium, iron, aluminium, potassium, sodium.

1. Petroleum treatment.—The powder was exhausted with petroleum. The soluble portion amounted to 1·25 per cent.; 0·5 per cent. was volatile oil and 0·75 per cent. was wax and a crystalline principle.

The crystals melt at 110° C. and are identical with those found in the root bark. The volatile oil is aromatic and pleasant, resembling in odor a mixture of bergamot and orange flower oils. The reactions are as follows: Strong sulphuric acid added to a chloroform solution gave a greenish color which changed to a purplish-red; ferric chloride and sulphuric acid gave a light green color, which gradually changed to brown and red brown.

2. Ether extraction.—The portion insoluble in petroleum spirit was treated with ether. The soluble portion amounted to 1·1 per cent., of which 0·2 were crystals and 0·9 resin. The extract is partly soluble in potassium hydrate and is precipitated on neutralization. The crystals have a bitter taste and the reactions obtained show it to be identical with the glucoside from the leaves.

3. Treatment with absolute alcohol.—The powder was next treated with absolute alcohol. The total extract amounted to 9·25 per cent., of which 6·125 per cent. was soluble in water. The glucoside was only found in the etherial solution as it existed only in a minute quantity.

4. Aqueous maceration.—The powder insoluble in the foregoing was treated with the requisite quantity of water; in this case double the quantity was used, making the proportion 1:20. The precipitate by alcohol amounted to 2·48 per cent. The total extract was 7·48 per cent.

5. Alkaline extraction.—The powder was next treated with 0·2 per cent. solution of sodium hydrate. The portion soluble amounted to 4 per cent., of which 1 per cent. was precipitated by alcohol and acetic acid to neutralization.

6. Treatment with diluted acid.—The insoluble powder from the last treatment was treated with diluted 2 per cent. hydrochloric acid. The total extract amounted to 2·6 per cent., about 1 per cent. was precipitated by ammonia and consisted mostly of iron. The remaining insoluble powder after bleaching amounted to 62·3 per cent.

Seeds.—The moisture of the seeds amounted to 7 per cent. and the ash 2·222 per cent., the solubilities of which were as follows:

Soluble in water	·334
Soluble in hydrochloric acid	1·444
Insoluble in either (silica)	·444
	2·222 per cent.

The qualitative analysis resulted as follows: Acids: sulphuric, hydrochloric and phosphoric; Bases: iron, aluminium, magnesium, potassium, sodium.

1. Petroleum maceration.—The powdered seeds were extracted with

petroleum. A portion of the liquid on evaporation left a residue amounting to 35·8 per cent. and consisting of fixed oil. This is bland, without odor, has the specific gravity 0·903, and is saponified by alcoholic and aqueous potassium hydrate solution. Sulphuric acid has no action on it; nitrous acid converts it into elaidin.

2. Ether extraction.—The powder was next treated with ether. The dried extract amounted to 1·3 per cent. It was a soft yellowish resinous mass of an acid reaction.

3. The alcohol maceration was lost entirely through an accident.

4. Aqueous treatment.—The powder was treated next with water. The soluble portion amounted to 1 per cent., which was completely precipitated by alcohol.

5. Alkaline maceration.—The powder insoluble in the above was macerated with 0·2 per cent. solution of sodium hydrate. The total extract amounted to 15·9 per cent., of which 6 per cent. was precipitated by acetic acid and alcohol.

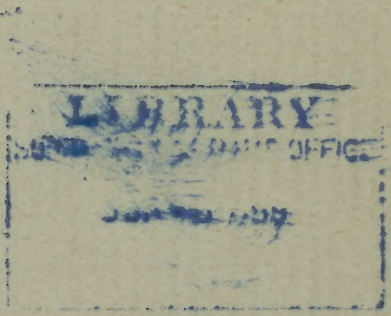
6. Acid extraction.—The powder was next treated with diluted 2 per cent. hydrochloric acid. The total extract amounted to 3 per cent.

The remaining insoluble powder was bleached and dried. It amounted to 31·4 per cent.

Recapitulation. Quantitative Results of Proximate Analysis.

	Leaves	Stem.	Root Bark.	Capsules.	Seeds.
Extracted by petroleum	2·600	·190	2·60	1·25	35·80
" " ether	1·460	·230	0·66	1·10	1·30
" " absolute alcohol	5·000	1·900	12·20	9·25	† .....
" " water	15·045	1·800*	8·90	5·000	1·00
" " diluted alkali	9·900	5·000	10·51	4·00	15·90
" " diluted acid	6·000	1·880	8·00	2·60	3·00
Residue (lignin)	42·600	64·005	42·625	62·300	31·40
Loss	3·645	14·829	·640	3·667	4·60
Moisture	13·750	10·166	13·865	10·833	7·00
Total	100·000	100·000	100·000	100·000	100·00
ASH.					
From air-dry part	5·033	1·333	5·700	3·500	2·222
From artificially dried	5·835	1·484	6·617	3·925	2·389

* Is not complete on account of loss. † This was entirely lost.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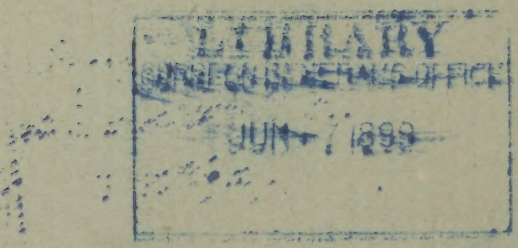
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